The *in-vitro* antibacterial activity of *Annona senegalensis* , *Securidacca longipendiculata* and *Steganotaenia araliacea* - Ugandan medicinal plants

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Abstract

Background: Nearly all cultures from ancient times to the present day have used plants as a source of medicines. As a result, different remedies tended to develop in different parts of the world. Current strategies to overcome the global problem of antimicrobial resistance include research in finding new and innovative antimicrobials from plants.

Objectives: To determine the antibacterial activity of extracts of *Annona senegalensis, Securidacca longipendiculata* and *Steganotaenia araliacea*, plants which are used in Eastern Uganda for the treatment of diarrhea and wound infections.

Methods: The root barks of these plants were collected, sun-dried, pounded using pestles and wooden mortars. Water and methanol extracts were derived and screened. Standard cultures of *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used in the study. The antibacterial tests used were the agar well diffusion assays. The minimum inhibitory concentrations (MIC) were determined by cylinder plate technique.

Results: Extracts of *Annona senegalensis, Steganotaenia araliacea* and *Securidaca longipendiculata* showed activity against *Staphylococcus aureus*. The methanol extract of *Securidaca longipediculata* was not inhibitory to *S.aureus*. *Steganotaenia araliacea* and *Securidaca longipediculata* (water) extracts were active against all the bacteria tested. The water and methanol extracts of *Annona senegalensis* did not show inhibitory activity against *E. coli*. Of the three bacteria studied, *Staphylococcus aureus* was the most susceptible to the extracts followed by *P. aeruginosa*. *E. coli* was least sensitive to the plant extracts. *Annona senegalensis* had the lowest MIC against *Staphylococcus aureus* (62.5mg/ml); while *Steganotaenia araliacea* had the highest MIC (250mg/ml) signifying lower activity.

Conclusions: The root bark extracts of the three plants showed antibacterial activity, justifying their continued use in treatment of bacterial infections.

Recommendations: Further studies are required to isolate and characterise the active phytochemical constituents in the plants. Toxicity studies should be done to determine their safety.

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Introduction

Preparations from plants were the original therapeutic interventions used by man to control diseases in humans and livestock. Nearly all cultures from ancient times to the present day have used plants as a source of medicines. Development of herbal products depended on local botanical flora. As a result, different remedies tended to develop in different parts of the world. In some instances, related plants were used over wide geographical regions as a result of communication or importation of plant materials of high repute[1]. There is now resurgence in interest in natural products as sources of novel compounds to combat the ever present threat of disease.

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Antimicrobial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antimicrobials[2]. Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant forms[3]. A considerable percentage of the peoples in both developed and developing nations use medicinal plant remedies. In the industrialised countries, consumers are seeking visible alternatives to modern medicine where there is already a problem of over-medication; accompanied by development of resistance to some[4]. In 1997, the 30th World Health Assembly adopted a resolution urging interested governments to utilize their traditional systems of medicine with regulations suited to their national health care systems.

Here we report the findings of a study that was designed to test the antibacterial activity of extracts from selected medicinal plants that are used in the Teso region of Eastern Uganda.

Materials and methods

Plant collection and pre-extraction preparation

The plants were chosen based on a feasibility study report on indigenous knowledge practices in the Teso region[5]. Vernacular names of plants [*Annona senegalensis* ['Ebwolo']; *Securidaca longipedunculata* ['Elilioi'] and *Steganotaenia araliacea* ['Ebusibusi'] were initially used to aid in the identification of these plants. Voucher specimens was prepared and taken to Makerere University herbarium in Kampala for identification. The roots of these plants were collected, sun-dried, pounded using pestles and wooden mortars and transported to the pharmacology research laboratory in the Faculty of Veterinary Medicine, Makerere University Kampala.

Extraction procedures

Annona senegalensis powder (200g) was soaked in 500ml of 99.5% methanol and placed on a shaker (Mitamura model-s102) for two hours. The sample was then strained using a tea strainer to remove solids. The resulting filtrate was further filtered using Whatman filter paper No. 1 to obtain a solution free of solids. The solution was then concentrated in a rotary evaporator (Heidolph-wb-200) to remove the methanol and 11.7g of the extract were obtained then stored at 2-4°C. *Securidaca longipedunculata* and *Steganotaenia araliacea* was extracted in the same way as above then 22.9g and 10.2g were obtained respectively. The same procedure was followed for water extracts except that; 200g of each sample was dissolved in 1000mls of distilled water and then concentrated by drying at 37°C. *Annona senegalensis*, *Securidaca longipedunculata* and *Steganotaenia araliacea* produced 12.1g, 20.3g, and 15.9g of the extract respectively. They were also stored at 2-4°C.

Preparation of bacteria

Mueller Hinton agar (35g) (Biotech UK) medium was mixed with one litre of distilled water, enclosed in a screw cap container and autoclaved at 121°C for 15min. The medium was later dispensed into 90mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37°C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile. Standard culture of *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 obtained from Mulago hospital clinical laboratories were chosen for the study. *E. coli* was used *to* represent the Gram-negative bacteria. *Pseudomonas aeruginosa* was used to represent opportunistic and multi-drug resistant bacteria. *Staphylococcus aureus* represented the Gram-positive bacteria.

Determination of antibacterial activity

The antibacterial activity screening was done as described before[6]. Briefly, cultures of the microorganisms from culture plates were scooped using a wire loop and separately mixed using normal saline and agitated with vortex mixer. A loop full was withdrawn and uniformly distributed on the surface of the agar plate by streaking using a sterile swab. Wells of approximately 4mm in diameter and 2.5mm deep were made on the surface of the solid medium using a sterile borer. The plates were turned upside down and the wells labeled with a marker. The extracts were reconstituted by dissolving 1gm of each in 1ml of dimethylsulfoxide (DMSO). Each well was filled with test sample. Sterile DMSO was used as negative control and disk of 10 microgram-equivalent Gentamicin was used as positive control. The plates were incubated at 37°C for 24hours. After 24hours the plates were removed and zones of inhibition measured with a pair of calipers and a millimeter ruler and the results tabulated. Extracts with zones of inhibition greater or equal to 6mm diameter were regarded as positive[7].

The minimum inhibitory concentration (MIC) of some of the positive extracts was determined by cylinder plate technique[8]. The extracts were reconstituted by dissolving 1g of each sample in 1ml of DMSO then subjected to a series of doubling dilutions up to the fifth stage in the test tubes containing 1ml sterilised normal saline. The extracts (0.5ml) were placed in the wells made on the culture media (containing the bacteria). The plates were then incubated for 18 hours at 37°C and zones of inhibition measured as above to determine the MIC.

Results

Antibacterial activity

All the plant extracts tested (*Annona senegalensis, Steganotaenia araliacea* and *Securidaca longipedunculata*) showed activity against *Staphylococcus aureus* except *Securidaca longipendiculata* methanol extract. Water extracts showed higher activity than methanol extracts. *Steganotaenia araliacea* and *Securidacca longipedunculata* (water) extracts were active against all the bacteria tested. The water and methanol extracts of *Annona senegalensis* showed little activity against *E. col.* Of the three bacteria studied, *Staphylococcus aureus* was the most susceptible to the extracts followed by *P. aeruginosa*. *E. coli* was least affected by the extracts.

Table 1: Antibacterial activity of the plant extracts expressed as Zone diameter (ZD) of inhibition of bacteria

Plant	Extract (1g/ml)	*S. aureus* Status	ZD(mm)	*E. coli* Status	ZD(mm)	*P. aeruginosa* Status	ZD(mm)
Annona	Water	A	18	N	?	N	?
	Methanol	A	16	N	?	A	8
Stegano	Water	A	10	A	7	A	12
	Methanol	A	13	N	?	A	8
Securida	Water	A	12	A	12	A	13
	Methanol	A	?	A	?	N	?

Key to table 1

Symbol	Meaning
A	Active
N	Not active
Annona	*Annona senegalensis*
Stegano	*Steganotaenia araliacea*
Securida	*Securidaca longipedunculata*

As can be seen in table 2, the minimum inhibitory zone increased with the increasing concentration of extracts. *Annona senegalensis* showed the lowest MIC against *Staphylococcus aureus* (62.5mg/ml) signifying higher activity while *Steganotaenia araliacea* showed the highest MIC (250mg/ml) signifying lower activity.

Table 2: Minimum inhibitory concentration (MIC) of the plant extracts on bacteria

Plant	Extract (1g/ml)	*S. aureus* MIC	ZD	*E. coli* MIC	ZD	*P. aeruginosa* MIC	ZD
Annona	Water	1000.0	18	?	?	?	?
	Methanol	62.5	10	?	?	?	?
Stegano	Water	1000.0	10	1000.0	7	1000.0	12
	Methanol	250.0	9	?	?	1000.0	8
Securida	Water	1000.0	12	1000.0	12	1000.0	13
	Methanol	?	?	1000.0	8	–	?

Key to table 2

Symbol	Meaning
MIC	Minimum inhibitory concentration (mg/ml)
ZD	Zone diameter (mm)
Annona	*Annona senegalensis*
Stegano	*Steganotaenia araliacea*
Securida	*Securidaca longipedunculata*

From concentration of 250mg/ml to 1000mg/ml, *Annona senegalensis* showed a range of 13 to 16mm while *Steganotaenia araliacea* showed a range of 9 to 13mm (Table 3).

Table 3: Relationship between concentrations of the plant extract with zone of inhibition on *Staphylococcus aureus* in determination of MIC

Concentration of plant Extract (mg/ml)	*Annona senegalensis* methanol extract ZD (mm)	*Steganotaenia araliacea* methanol extract ZD (mm)
1000	16	13
500	15	12
250	13	9
125	12	?
62.5	10	?
31.25	?	?

Key to table 3

Symbol	Meaning
ZD	Zone diameter

S. aureus was the most susceptible to the positive control drug with a zone diameter (ZD) 17mm while *E. coli* and *P. aeruginosa* showed the same ZD of 16mm. The negative controls, normal saline and DMSO did not show any activity (Table 4)

Table 4: Zone diameter (of) Positive and negative controls

Control	*S. aureus*		*E. coli*		*P. aeruginosa*	
	Status	ZD (mm)	Status	ZD (mm)	Status	ZD (mm)
Gentamicin+	A	17	A	16	A	16
DMSO ?	N	?	N	?	N	?
Normal saline ?	N	?	N	?	N	?

Key to table 4

Symbol	Meaning
+	Positive control
A	Active
N	Not active
?	Negative control

Discussion

All plants studied showed antibacterial activity. This could justify their use in treatment of microbial infections in man and livestock. Water extracts showed higher activity compared to methanol extracts on bacteria. This may be as a result of failure of the methanol to extract the active ingredient; water is a more polar solvent than methanol. *Steganotaenia araliacea* and *Securidaca longipedunculata* water extracts were active against all the bacteria tested. These plants could be effective and suitable for general use against Gram negative, Gram positive and opportunistic pathogens of humans.

Annona senegalensis extracts did not have measurable activity against *E.coli*. Therefore it may not be of value in treatment of infections caused by *E. coli* such as diarrhoea and urinary tract infections. It may not also be useful in treatment of other gram-negative bacterial infections. However, UTI can be caused by *P. aeruginosa* or *S. aureus* against which *Annona senegalensis* was active. This justifies its use in treatment of UTI in animals as reported by Katende *et al.*, (1995). *S. aureus* was the most susceptible to the plant extracts. This could support the use of these plants in treatment of Staphylococcal infections. *Annona senegalensis* could be of value for UTI[9] and *Securidaca longipedunculata* in the treatment of post traumatic infections[10].

The lack of susceptibility of *Pseudomonas aeruginosa* to the extracts could be attributed to the fact that this bacteria are naturally resistant to many antibiotics due to the permeability barrier afforded by its outer

membrane[11]. Also its tendency to colonise in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Since its natural habitat is the soil, living in association with bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally occurring antibiotics[11].

The lack of susceptibility of the bacteria to the plant extracts against *Annona senegalensis* could be attributed to the fact that, unlike conventional pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from materials of plant origin which may be subjected to contamination and deterioration[12]. The storage of extracts may require special condition of humidity or temperature or protection from light. The plant extracts might contain little of the active ingredient. The extracts which were inactive *in-vitro* may have properties similar to pro-drugs which are administered in an inactive form; their metabolites could be active *in-vivo*[13]. From findings reported elsewhere, the antibacterial activity of the plants could be due to the following chemicals: Senegalene[14] and roemerine[15] from *Annona senegalensis*. Elymoclavin, dehydrelymoclavine, benzoic acid derivatives and ergot alkaloids from *Securidaca longipedunculata*. Polyacetylenes, arthoquinolones, polyacetates, sucrose and facorinone[16] from *Steganotaenia araliacea*.

Conclusions and recommendations

Water and methanol crude extracts of *Annona senegalensis*, *Securidaca longipedunculata* and *Steganotaenia araliacea* were obtained and used in this study. The demonstration of antibacterial activity in the extracts would seem to justify their use in treatment of microbial infections. *Annona senegalensis* showed the lowest MIC against *staphylococcus aureus* (62.5mg/ml) signifying higher activity while *Steganotaenia araliacea* showed the highest MIC (250mg/ml) signifying lower activity. *Steganotaenia araliacea* and *Securidaca longipedunculata* (water) extracts was active against all the bacteria tested. The findings suggest that there is potential in discovering novel antimicrobial agents from indigenous medicinal plants that are used in this country. This potential needs further investigation.

From the findings of this study, the following recommendations could be made. Firstly, studies should be done in order to identify the active phytochemical constituents and evaluate their effectiveness *in-vivo* so that they can be synthesised commercially. Secondly, there is need for *in-vivo* trials to identify those plants that are effective and suitable for general use. Studies to determine drug-drug or drug – food interactions should also be done. This is vital for a drug to be considered useful. Finally, toxicity studies of the active plants should be done to determine their safety.

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